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Original Research

The efficacy of olive leaf extract on healing herpes simplex virus labialis: A randomized double-blind study

Tahereh Toulabi[a,b,*], Bahram Delfan[a,d], Marzieh Rashidipour[a], Sajad Yarahmadi[b,c], Farzaneh Ravanshad[b], Amir Javanbakht[e], Mohammad Almasian[d]

[a] Razi Herbal Medicines Research Center, Lorestan University of Medical Sciences, Khorramabad, Iran
[b] School of Nursing & Midwifery, Lorestan University of Medical Sciences, Khorramabad, Iran
[c] Nursing Care Research Center, Semnan University of Medical Sciences, Semnan, Iran
[d] School of Medicine, Lorestan University of Medical Sciences, Khorramabad, Iran
[e] Lorestan University of Medical Sciences, Khorramabad, Iran

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ABSTRACT

Objective: Herpes simplex virus (HSV), as a common infection in healthy individuals, is treated symptomatically, but drug resistance and the side effects of drugs have drawn the attention of researchers to complementary medicine. Olive Leaf Extract (OLE) has antiviral effects that may treat HSV. The current study aimed to compare the clinical effects of OLE and Acyclovir on HSV-1.
Methods: This randomized double-blind clinical trial was conducted on 66 patients who had already been diagnosed with HSV-1. The participants were randomized into two groups, receiving 2% OLE cream or 5% acyclovir cream five times a day for six days. The symptoms were evaluated before, and three and six days after the interventions. Data were analyzed using the SPSS software through the Kolmogorov-Smirnov test, chi-squared, t-test, and repeated measures ANOVA.
Results: The results showed clinical symptoms decreased in both groups during the study and both medications were effective in the treatment of HSV-1. However, the OLE group experienced less bleeding ($P = 0.038$), itching ($P = 0.002$), and pain ($P = 0.001$) on the third day as well as less irritation ($P = 0.012$), itching ($P = 0.003$) and color change ($P = 0.001$) on the sixth day compared to the acyclovir group. The treatment course for participants in the OLE group was shorter than in the acyclovir group ($P = 0.001$).
Conclusion: The evidence from these trials suggests the OLE cream is superior in the healing of episodes of HSV-1 over the acyclovir cream. Future studies are recommended to investigate if OLE could be an adjunct to acyclovir treatment.

Introduction

Herpes simplex virus (HSV) is one of the most recurrent and commonest skin diseases. Based on estimates from 2012, more than 3.7 billion individuals younger than 50 are infected with HSV.[1,2] Eighty percent of the American population is exposed to HSV and all age groups can be affected.[3] Although both HSV-1 and HSV-2 affect the skin and mucosal tissues, the oral and facial areas are most commonly affected by HSV-1. Symptoms of HSV-1 infection include painful lip vesicles that combine and grow to ulceration, lasting for almost 12 days accompanied by general symptoms like fever, malaise, and dryness.[4] Antiviral drugs including acyclovir, ganciclovir, valaciclovir, and penciclovir are used to treat HSV-1. These drugs prevent viruses from multiplying and, as a result, hinder the emergence of severe symptoms of herpes and reduce the chances of transmission.[5]

Nucleoside analogues have partially been successful in curing HSV infection; however, their use leads to viral resistance and therefore viral latency and recurrence.[6] On the other hand, these drugs are expensive, all patients are not able to afford them in the long term due to the recurrence of HSV.[7] Therefore, researchers are looking for an effective and inexpensive medication with the fewest side effects to reduce the severity of HSV, shorten its duration, reduce its infectivity, and prevent its recurrence as much as possible. Numerous complementary and alternative medicines for HSV prevention and treatment have been suggested. They include Aloe vera, topical zinc, Melissa officinalis, propolis, Astragalus, Vitamin C, Echinacea, cat's

Institute: Razi Herbal Medicines Research Center, Lorestan University of Medical Sciences, Khorramabad, Iran.
* Corresponding author at. Razi Herbal Medicines Research Center, Lorestan University of Medical Sciences, km 5 Road of Khorram Abad - Boroujerd - opposite to the Kahriz village, Postal code: 6816885916, Khorramabad, Iran.
E-mail addresses: Tolabi.t@lums.ac.ir (T. Toulabi), Delfanb@yahoo.com (B. Delfan), m.rashidipour@lums.ac.ir (M. Rashidipour), S.Yarahmadi000@gmail.com (S. Yarahmadi), fr.ravanshad@gmail.com (F. Ravanshad), javanbakht7@yahoo.com (A. Javanbakht), almasian@yahoo.com (M. Almasian).

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claw, elderberry, sandalwood, sage-rhubarb, tea tree oil, Eleutherococcus, kelp, witch hazel, and others.[2]

Recently, olive leaves began to gain special consideration. In European and Mediterranean countries, olive leaves are processed into an extract and used in traditional remedies. Iranians use olive leaves for the treatment of wounds and skin diseases.[8] It is well known that oleuropein and its derivatives such as hydroxytyrosol and tyrosol are the main phenolic constituents of olive leaves and are thought to be responsible for the pharmacological effects of olive leaves.[9] Studies have reported that olive leaf extract (OLE) has antiviral[10] antioxidant,[11] inhibitory effects on candida growth,[12] antifungal[13] anti-inflammatory[14] and antibacterial activities.[15] Newly, in an American patent, OLE has been confirmed to have a strong antiviral impact against the hepatitis virus, herpes mononucleosis, bovine rhinovirus, canine parvovirus, rotavirus, respiratory syncytial virus, parainfluenza type 3 viruses, and feline leukemia virus.[10] Molecular studies have also shown that OLE has antiviral effects against HIV-1.[16] The results of an *in-vitro* study indicate that a standardized ethanolic extract of the dry olive leaves (70% V/V) inhibits HSV-1. The findings of this study indicated that the hydroxytyrosol found in OLE has significant antiviral activity against HSV-1[8] Since an extensive search in related studies did not yield any results about trials on the effect of OLE on the treatment of HSV-1, this study was conducted to examine the impact of a 2% OLE cream on the healing of HSV-1 and to compare it with a 5% acyclovir cream.

Material and methods

Study design and participants

This was a randomized double-blind, parallel-group, single-center, controlled trial. The study population included all patients who had already been diagnosed with HSV-1 and were selected using the convenience sampling method. The study was performed at the Dermatological Clinic of the Lorestan University of Medical Sciences, Khorramabad, Iran from September 2015 through February 2016. The patients were examined by a dermatologist to confirm the presence of orofacial HSV-1 infection. HSV-1 was diagnosed based on symptoms including pain, itching, irritation, bleeding, and discoloration.

Inclusion and exclusion criteria

The inclusion criteria consisted of: (1) an age range of 18–70 years in both genders, (2) having HSV-1 in the orofacial region if they had observable blisters (second stage) for no more than thirty hours before the examination. Participants were excluded if (1) they had a history of allergy to antiviral drugs, (2) they suffered from immunosuppressive diseases, (3) they had used antiviral medication two weeks ago, (4) HSV-1 was serious requiring systemic treatment, (5) they used the studied drugs irregularly, (6) they were unwilling to continue taking the medications, and (7) in cases of drug hypersensitivity or complications.

Interventions

Participants were assigned randomly to a 2% OLE group and a 5% acyclovir (ALOVIR®, Parsdarou, Iran) group with a ratio of 1:1. The subjects were asked to apply the drugs topically to ulcerous areas five times a day for six days. The daily dose corresponded to approximately 1 gr of Acyclovir cream and approximately 0.4 gr of OLE cream. To confirm drug adherence, patients were contacted daily to be reminded them of using the medication during the treatment period. Patients were visited as follow-up after three months and asked about the recurrence of HSV

Plant material

The olive leaf samples (Olea europaea, a variety of Sevillano, family of Oleaceae) were collected in February from Poledokhtar, Iran. The plant sample was identified by an herbalist and a voucher specimen was kept at Razi Herbal Medicine Research Center, Khorramabad, Iran. Olive leaf was dried at room temperature away from the sun and was ground to powder utilizing an electrical mill. Each 1 g of the powder was percolated with 10 mL ethanol (70% v/v) in water and it was stirred for 12 h in two stages. The extracts were evaporated and dried with a rotary evaporator and then were washed with dichloromethane and methanol solutions with a 98:2 ratio. The insoluble parts were removed and completely dried using a freeze dryer. Oleuropein (C25H32O13) was detected using a High-Performance Liquid Chromatography (HPLC) device with an isocratic washing program with a strontium solvent and a 50 mM phosphate buffer at a pH of 2.9 with a ratio of 70:30 (v:v). HPLC showed that oleuropein (356 mg/g), hydroxytyrosol (4.89 mg/g) and tyrosol (3.73 mg/g) were the main phenolic compounds of the OLE.

Preparation of the cream

A topical cream containing 2% OLE was prepared using an Ucerin base. The Ucerin was melted at 65–70 °C; then, it was cooled to 40 °C, other components of the formulation were added to it. The formulation contained 10% propylene glycol, 2% OLE, and 0.1% ascorbic acid. To ensure that the participants and our colleagues cannot identify the type of cream, the appearance and odor of the OLE cream were designed to match that of the acyclovir cream.

Outcome evaluations

Clinical examinations took place before the intervention, and 3 and 6 days after the intervention. The frequency of incidence of clinical symptoms including irritation, itching, bleeding, color change, and pain was evaluated by a dermatologist and recorded in a questionnaire. In addition, symptom healing time was assessed. The ulcer size was measured with a disposable folding ruler and a photo was taken from the ulcer using Dino-Lite Digital Microscope AM313.

Randomization method

Participants were assigned randomly to the OLE group (n = 33) and the Acyclovir group (n = 33) using block randomization. Random numbers based on the allocation sequence were generated using the Statistical Analysis System (SAS; Version 8.7). A biostatistician designed the random allocation procedure and enrolled participants.

Blinding method

Study medications were filled into identical tubes containing 10 mL of each cream. The creams were indistinguishable in terms of outward appearance and consistency. The tubes were coded confidentially and drug codes remained with a biostatistician until the end of the analysis. This procedure assured full blinding of researchers, the dermatologist, and participants.

Data analysis

A sample size of 33 participants in each group was selected. Qualitative data are shown as numbers and percentages. Quantitative data are shown as means and standard deviations. The Kolmogorov-Smirnov test evaluated the normality of the variable. Descriptive statistics were used to organize and summarize the demographic data. A paired t-test was used for statistical evaluation of normal distribution data. The chi-square analysis was utilized for the statistical assessment of qualitative

variables. Repeated-measures ANOVA was employed to compare longitudinal data. Data analysis was performed using SPSS version 22. *P*-values less than 0.05 were considered as statistically significant.

Results

A total of 66 patients was enrolled into the two groups (33 patients in each group). Two subjects in the OLE group were excluded from the study for irregular use of the drug. Moreover, two subjects in the acyclovir group were excluded after enrollment, because they needed systemic treatment. Finally, sixty-two patients remained in the study (Fig. 1). The mean age of participants was 34.90 ± 11.22 years. Female participants made up 61.29% of the study population. No statistically significant differences were observed before the interventions between groups in terms of age, gender, marital status, education, occupation, frequency of HSV-1 recurrence per year, location, and pain intensity ($P > 0.05$, Table I). However, the ulcer size was larger ($P = 0.035$, Table 1) and the irritation ($P = 0.030$, Table 3) was significantly more intense in the OLE group before the interventions. The results showed the frequency of symptoms were significantly reduced over the follow-up period in both groups ($P > 0.05$, Table 2).

On the third day, significantly fewer participants in the OLE group had bleeding (6.5% vs 25.8%, $P = 0.038$), itching (12.9% vs 48.4%, $P = 0.002$) and severe pain (3.2% vs 35.5%, $P = 0.001$) compared to the acyclovir group. Additionally, results indicated that on the sixth-day, significantly fewer participants in the OLE group had irritation (3.2% vs 25.8%, $P = 0.030$), itching (3.2% vs 32.3%, $P = 0.003$) and color change (12.9% vs 61.3%, $P = 0.001$) compared to the acyclovir group (Table 3). The healing time in the OLE group was significantly shorter than in the acyclovir group (2.78 ± 2.15 vs 5.03 ± 135, $P = 0.001$). Moreover, the OLE group had significantly shorter healing times to resolution of clinical symptoms (Table 4). The number of healed patients in the OLE group were significantly higher in the third (90.3% vs 51.6%, $P < 0.001$) and sixth (93.5% vs 58.1%, $P < 0.001$) days compared to the acyclovir group. Fig. 2 and Fig. 3 show photos taken of two participants of the OLE and acyclovir groups. Three months after the start of the intervention, participants were followed up to evaluate the recurrence of HSV-1 and no recurrence was observed in both groups. Side effects reported in the OLE group included 7 cases of a slight local irritation while applying the cream. Due to the mildness of this complication, none of the participants stopped using the cream nor did they withdraw from the study.

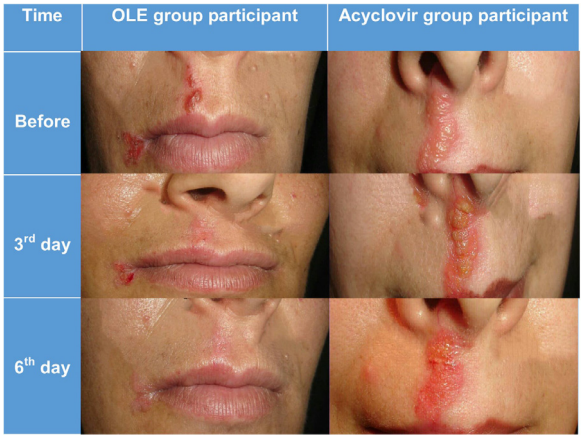

Fig. 2. Photographs of the healing process of two study participants.

Discussion

This clinical trial was conducted to compare the effects of 2% OLE cream and 5% acyclovir cream on the healing of HSV-1. The results

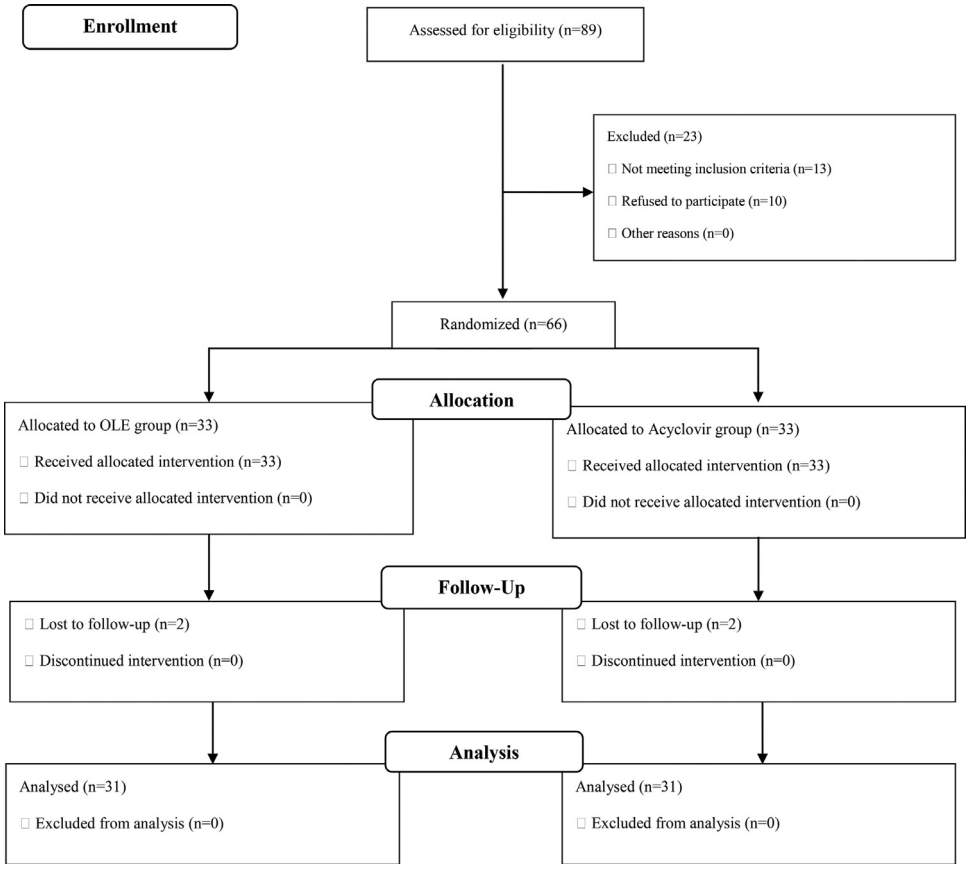

Fig. 1. Flow diagram of the study.

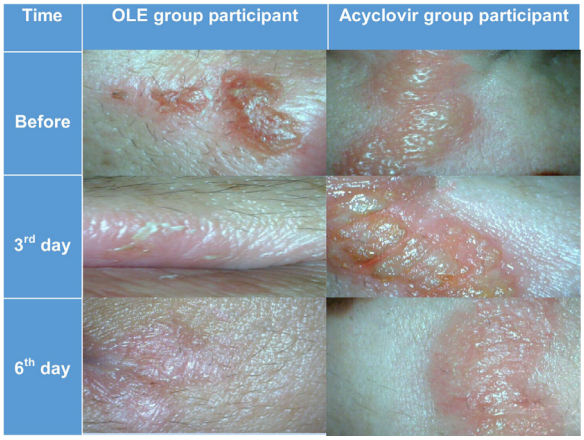

Fig. 3. Digital Microscope photographs of the healing process of two study participants.

showed that both groups exhibited improved HSV-1 with a decreasing trend in clinical symptoms. However, lower frequencies of itching, bleeding, and pain were observed in the OLE group on the third day. OLE was also more efficient in controlling irritation, itching, and color change as compared with acyclovir until the sixth day. The number of healed patients in the OLE group was significantly higher in the third and sixth days compared to the acyclovir group.

The anti-HSV-1 activity of OLE might be attributed to components of olive leaves including oleuropein, olenolic acid, hydroxytyrosol, or calcium elenolate.[17] The main ingredient in OLE is oleuropein, an anti-inflammatory agent,[18] and it has been claimed to have strong antiviral activities against the hepatitis virus, herpes mononucleosis, bovine rhinovirus, canine parvovirus, rotavirus, and feline leukemia virus. Oleuropein is known as an antioxidant that can reduce cellular damage to a minimum allowing the improvement of wound healing.[18,19] Hydroxytyrosol also has antioxidant and anti-inflammatory properties and has anti-HIV activity.[20] An *in-vitro* study compared the antiviral effect of OLE and acyclovir, the results of which showed oleuropein has high anti-HSV activity similar to acyclovir.[21] In another study, it was reported that the antiviral activity of OLE might be ascribed to the inhibition of attachment and sorption of virus particles to the cell, which blocks their access to cells.[10]

Table 1
Comparison of baseline and demographic characteristics among the study groups.

Variables	OLE group N (percent)	Acyclovir group N (percent)	*P*-value
Age*	36.1 ± 13.4	33.6 ± 8.7	0.390
Gender			
Female	18(58.06)	20(64.51)	0.602
Male	13(41.93)	11(35.48)	
Marital status			
Single	11(35.48)	9(29.03)	0.607
Married	16 (51.61)	14(45.16)	
Divorced	1(3.22)	3(6.66)	
widow	3(9.67)	5(16.12)	
Education			
Illiterate	1(3.22)	2(6.44)	0.584
Under Diploma	6(19.35)	10(32.25)	
Diploma	12(38.70)	9(29.03)	
University	12(38.70)	10(32.25)	
Employment Status			
Unemployed	6(19.35)	9(29.03)	0.452
Housewife	14(45.16)	8(25.80)	
Working	10(32.25)	13(41.93)	
Retired	1(3.22)	1(3.22)	
Location			
Urban	24(77.41)	18(58.06)	0.103
rural	7(22.58)	13(41.93)	
Mean indication of HSV per year (range)	4.9 (1–7)	3.3 (1–5)	0.101
Ulcer size (mm^2)*	5.8 ± 1.10	3.60 ± 0.61	0.035

Age and ulcer size based on mean and standard deviation (Mean±SD)

Table 2
Comparison the frequency of symptoms and ulcer size during the study in the participants.

Clinical symptoms	Before	Third day	Sixth day	*P*-value
OLE group				
Irritation	2.75± 2.25	1.67 ± 1.83	1.58 ± 1.57	0.001
Bleeding	2.19 ± 1.92	1.90 ± 2.22	1.90 ± 1.97	0.011
Itching	2.63 ± 2.39	1.76 ± 1.85	1.61 ± 1.61	0.001
Color change	2.48 ± 2.47	2.21 ± 2.39	1.30 ± 1.24	0.001
Pain	2.58 ± 2.71	1.70 ± 2.08	1.70 ± 1.67	0.001
Ulcer size (mm^2)	5.8 ± 1.10	6.0 ± 3.60	2.6 ± 2.60	0.001
Acyclovir group				
Irritation	2.30 ± 2.45	1.87 ± 1.82	1.83 ± 2.01	0.006
Bleeding	2.03 ± 2.00	2.16 ± 2.18	1.83 ± 1.83	0.016
Itching	2.29 ± 2.23	1.97 ± 2.08	1.75 ± 1.34	0.002
Color change	2.26 ± 2.47	2.03 ± 2.20	1.70 ± 1.74	0.001
Pain	2.35 ± 2.27	2.00 ± 1.3	1.67 ± 1.32	0.001
Ulcer size (mm^2)	3.60 ± 0.61	4.70 ± 3.40	2.50 ± 2.60	0.003

Antiviral effects of the special OLE applied in this trial have been demonstrated in several studies.[10,22,23] The acyclovir lip cream is currently the gold standard for the treatment of HSV-1.[24] The effectiveness and safety of acyclovir is not doubted and confirmed in this study and previous studies.[24,25] However, we have found a clear superiority of OLE over the reference acyclovir lip cream. HSV-1 is a self-limiting condition and would usually heal within ten to fourteen days, if not treated.[26] The treatment aim is to reduce this period, and a reduction of the HSV-1 episode was confirmed for both the OLE and acyclovir groups. However, the healing period was shorter in the OLE group than in the acyclovir group. The results of another study showed that the use of 0.5% propolis) drug extract ratio 2:1, extraction solvent ethanol; excipients in the lip balm) in the treatment of HSV is more effective than 0.5% acyclovir.[26] Other natural compounds have included an aqueous dry extract of the sage leaf (*Salvia officinalis* L.) and a standardized ethanolic (70%V/V) dry extract of rhubarb (*Rheum palmatum* L. or Rheum officinale Baillon), which have effects like the acyclovir cream.[27]

Lemon balm (active ingredient: 1%, dried extract from *Melissa officinalis* L. leaves) was more effective in treating HSV than placebo and it could shorten the healing time, prevented the spread of the infection and had quick effects on had symptoms, [28] but largely failed when compared to acyclovir.[29] Likewise, the results of only one study on Aloe vera extract (0.5% *Aloe barbadensis Mill* was added into a hydrophilic cream) suggest that Aloe vera extract is more efficacious than placebo in treating the first episodes of genital herpes in men.[30] Melissa gel (active ingredient: 1%, dried extract from *Melissa officinalis* L. leaves) effectively reduced pain severity on the second and fourth days but it was not effective in treating recurrent HSV-1.[29] A review on the subject established that L-lysine may be effectual in virus prevention.[31] There is some evidence that vitamin C (ascorbic acid)[32,33] and tea tree oil (*Melaleuca alternifolia*) are useful in the treatment of HSV,[34] while other natural substances such as *Echinacea purpurea* (800 mg of *E. purpurea* extract, plant 95% and root 5%) was found not to be useful.[35] It has been stated in another study that the dermal application of zinc lotions or anti-inflammatory and antiseptic topical agents is not entirely satisfactory.[36]

Every herb may contain hundreds or thousands of chemicals and may exert its pharmacological effects by affecting multiple targets and pathways. Additionally, an herb's properties may be affected by such aspects, as the specific species applied, harvest time and situations of growth (exposure to sunlight, climate, soil, etc.), the part of the plant used (root, stem, leaf, etc.), processing (washing, drying, steaming, etc.), and conditions while in storage (humidity, temperature, etc.). As these aspects vary, they can lead to differences in the chemical compounds of herbs from batch to batch and may result in

Table 3
Comparison of clinical symptoms of patients with herpes in study groups.

Clinical symptoms	OLE group Yes N (percent)	No N (percent)	Acyclovir Yes N (percent)	No N (percent)	*P*-value
Irritation before intervention	25 (80.6)	6 (19.4)	17 (54.8)	14 (45.2)	0.030
Irritation on the third day	3 (9.7)	28 (90.3)	8 (25.8)	23 (74.2)	0.096
Irritation on the sixth day	1 (3.2)	30 (96.8)	8 (25.8)	23 (74.2)	0.012
Bleeding before intervention	8 (25.8)	23 (74.2)	5 (16.1)	26 (83.9)	0.349
Bleeding on the third day	2 (6.5)	29 (93.5)	8 (25.8)	23 (74.2)	0.038
Bleeding on the sixth day	2 (6.5)	29 (93.5)	1 (3.2)	30 (96.8)	0.554
Itching before intervention	22 (71)	9 (29)	20 (64.5)	11 (35.5)	0.587
Itching on the third day	4 (12.9)	27 (87.1)	15 (48.4)	16 (51.61)	0.002
Itching on the sixth day	1 (3.2)	30 (96.8)	10 (32.3)	21 (67.7)	0.003
Color change before intervention	29 (93.5)	2 (6.5)	30 (96.8)	1 (3.2)	0.554
Color change on the third day	23 (74.2)	8 (25.8)	25 (80.6)	6 (19.4)	0.544
Color change on the sixth day	4 (12.9)	27 (87.1)	19 (61.3)	12 (38.7)	0.001
Pain before intervention	19 (61.3)	12 (38.7)	17 (54.8)	14 (45.2)	0.607
Pain on the third day	1 (3.2)	30 (96.8)	11 (35.5)	20 (64.5)	0.001
Pain on the sixth day	1 (3.2)	30 (96.8)	4 (12.9)	27 (87.1)	0.162

significant variance in the pharmacology of the herb. The try to identify a single component as the active compound in herbal medicine may disregard the synergistic activity of all the components and not take into account the role of other compounds. Methods have been recommended for the general evaluation of herbs, which try to present a comprehensive profile of herbs or the chemical fingerprint.[37] Chromatographic fingerprinting of OLE is required to determine the phytochemical properties of herbs for further research and to verify therapeutic efficacy.

In summary, OLE can be a complementary treatment for HSV-1 or an alternative to acyclovir. No adverse reactions or toxicity reports have been documented, and no drug interactions are yet known.[38] However, the only unwanted side effect observed in this study was a slight local irritation while using OLE cream in seven patients. This seems to be due to the ingredients added to the OLE. The results of this study showed OLE was safe and well-tolerated by most participants.

Limitations

This is the first study to investigate the beneficial effects of OLE in the treatment of HSV-1. There were some limitations in this study such as a relatively small number of patients, and we did not perform serological tests to confirm the diagnosis of HSV-1. The lack of a placebo may be considered a weakness in the study design. However, the use of a placebo in this trial is considered immoral by the authors. We only used the 2% dose of OLE and other doses were not applied in this experiment. It is recommended that other doses be evaluated in other studies. Since herpes is a recurrent disease, it is suggested that the long-term efficacy of OLE be assessed in future studies.

Conclusion

This study confirms the clinical efficiency of use as well as a quick onset of effects of 2% OLE cream on HSV-1, with the clinical usefulness now established. OLE as an active pharmaceutical element of topical medications for the treatment of HSV-1 may, therefore, be of special importance for patients for whom the existing standard treatment is either not obtainable or not tolerated.

Table 4
Comparison of clinical symptoms during the treatment period between study groups.

Symptoms	OLE group Mean±SD	Acyclovir group Mean±SD	*P*-value
Irritation	2.03 ± 0.64	3.22 ± 1.45	<0.0001
Bleeding	1.68 ± 0.69	2.22 ± 1.36	0.0268
Itching	2.06 ± 1.13	3.90 ± 1.61	<0.0001
Color change	4.54 ± 1.38	5.93 ± 035	<0.0001
Pain	1.64 ± 1.17	2.87 ± 1.86	0.0017

Statement of ethics

Ethical approval of this research (No: LUMS.REC.2009.896) was obtained from the Ethics Committee of the Lorestan University of Medical Sciences, Khorramabad, Iran. The study was recorded in the Iranian Registry of Clinical Trials under the code of IRCT138808092652N1. Patients were informed about the purpose and the course of the study and that they were free to withdraw at any stage. Patients were assured about the confidentiality of the data and the absence of any constraints to participate. They were also asked to complete a written informed consent form before enrolling in the study.

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Author contributions

Study concept and design: Tahereh Toulabi, Bahram Delfan; acquisition of data, analysis and interpretation of data: Tahereh Toulabi, Marzieh Rashidi, Farzaneh Ravanshad; administrative, technical, and material support: Bahram Delfan, Amir Javanbakht; translation and editing: Mohammad Almasian, Sajad Yarahmadi.

Declaration of Competing Interest

The authors declare that there is no conflict of interest.

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